SUMMARY OF INVENTION

[009] The present invention provides a method for transferring and expressing heterologous DNA in a non-plant host cell. The vector used in this method, called BIBAC vector, includes a backbone having a first origin of replication capable of maintaining heterologous DNA as a single copy in an *Escherichia coli* host cell. The vector further includes a unique restriction endonuclease cleavage site for insertion of heterologous DNA, and left and right *Agrobacterium* T-DNA border sequences flanking the unique restriction endonuclease cleavage site. In certain host cells, the T-DNA border sequences allow introduction of heterologous DNA located between the left and right T-DNA border sequences into a host cell. In preferred embodiments, the vector includes a second origin of replication capable of maintaining heterologous DNA as a single copy in a host cell such as *Agrobacterium* species or other prokaryotic cells.

[0010] The method of the present invention allows for the construction of genomic libraries with large DNA inserts. The ability to transfer and express large segments of DNA increases the likelihood of cloning a cluster of genes that comprises an entire pathway.

[0011] Individual clones can be directly introduced into non-plant host cells by transformation. As used herein, "transformation" or "transforming" means introducing the DNA into the host cell by any appropriate means known in the art. For example, electroporation, calcium phosphate, triparental mating, particle bombardment. Transformation further includes *Agrobacterium*-mediated transformation.

[0012] Non-plant host cells include for example, prokaryotic organisms, filamentous fungi, yeast, insect, and mammalian host cells.

[0013] The method of the present invention, by providing for transfer and expression of large segments of DNA in diverse non-plant host cells, advantageously allows one to screen the clones, e.g., a genomic library, for expression of the desired gene product or products in a variety of conditions. For example, using the method of the present invention, a genomic library can be screened for the expression of a desired gene product, e.g. an antibiotic. If the gene product is found not to be expressed in *E. coli*, the genomic library can then be expressed in another prokaryotic host cell, for example, *Agrobacterium tumefaciens*. Since *Agrobacterium*, as a soil bacterium is adapted for growth at lower temperatures than *E. coli*, it may be better suited for expression of certain gene products e.g., enzymes from microorganisms that prefer to grow in temperatures lower than *E. coli*. Additionally, the genomic library can further be screened for expression of a desired gene product in a eukaryotic host cell, such as yeast, filamentous fungi and mammalian host cells. Different host species will allow for the synthesis or degradation of different products as a result of differences in their physiological makeup. For example different species have different biochemical pathways. These features underscore the importance of the BIBAC vector in "gene prospecting" i.e. discovery, expression, and production of novel pathways. As well as its use in identifying DNA which encodes genes that results in the production or degradation of important compounds, the BIBAC vector can potentially be used for expression of the DNA for production of the useful compounds in commercial quantities.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] These and other features and advantages of this invention will be evident from the following detailed description of preferred embodiments when read in conjunction with the accompanying drawings in which:

[0015] Fig. 1 shows the map of the BIBAC vector; and

[0016] Fig. 2 shows the strategy for construction of the BIBAC vector.

DETAILED DESCRIPTION

[0017] The present invention relates to a method for transferring heterologous DNA into a non-plant host cell. The vector used in this method, designated BIBAC (binary bacterial artificial chromosome vector), includes a backbone having an origin of replication that is capable of maintaining heterologous DNA as a single copy in a bacterial host cell. As used throughout this application, unless otherwise indicated, maintenance as a single copy refers to a non-replicating cell, i.e. a cell not undergoing cell division; during cell division, the copy per cell increases to nearly two complete copies per cell. In certain preferred embodiments the vector includes a second origin of replication capable of maintaining the heterologous DNA as a single copy in an *Agrobacterium tumefaciens* host cell and in related species. Other origins of replication can be included depending on the desired host cell, if it is desirable to maintain the BIBAC in the host cell rather than transferring the T-DNA into the host's DNA.

[0018] The vector also includes a unique restriction endonuclease cleavage site for the insertion of heterologous DNA. The presence of only one cleavage site for a particular restriction endonuclease within the DNA sequence encoding the vector is the presence of a "unique" restriction endonuclease cleavage site. The particular restriction endonuclease will, therefore, only cleave the DNA at that one location or "unique site".

[0019] Heterologous DNA refers to DNA not normally present in the particular host cell transformed by the vector. Heterologous DNA can be obtained from different sources such as prokaryotes; different species such as mammalian, reptile, bird, amphibian, fish etc.; yeast;